to determine how these processes are involved during long-lasting depolarizations of isolated mouse muscle fibers under voltage control by measuring cytosolic Ca^{2+} changes using fura-2 or luminal SR Ca^{2+} changes using fluo5N in the presence of 50 mM internal EGTA. Decays of cytosolic Ca^{2+} signals elicited by 50-s duration depolarizations became more marked and faster with depolarization amplitude. Pre-depolarizations of 2-min duration and of increasing amplitude induced a reduction of voltage-activated cytosolic Ca^{2+} signals with a mean voltage of −50 mV inducing half-maximum reduction. A comparable protocol applied to fibers loaded with fluo5N showed that low voltage depolarizing prepulses induced a marked SR Ca^{2+} depletion that contributed to reduce a subsequent voltage-activated SR Ca^{2+} change with a mean voltage of −50 mV inducing half-maximum reduction. Measuring SR Ca^{2+} changes in response to long-lasting depolarizations indicated that SR Ca^{2+} release channels inactivated in response to much higher depolarizations with a mean half-maximum inactivation voltage of −20 mV. Finally, trains of action potential of 50 s duration produced cytosolic Ca^{2+} signals that decayed with time, whereas SR Ca^{2+} changes did not display any sign of inactivation. These results indicate that the decline in SR Ca^{2+} release during long-lasting depolarizations mainly results from SR Ca^{2+} depletion. The work was supported by AFM, CNRS and University Lyon 1.

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STIM1 Negatively Regulates the Ca^{2+} Release from the Sarcoplasmic Reticulum in Skeletal Myotubes

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Stromal interaction molecule 1 (STIM1) mediates store-operated Ca^{2+} entry (SOCE) in skeletal muscle. However, the direct role(s) of STIM1 in the innate skeletal muscle event such as the Ca^{2+} release from the sarcoplasmic reticulum (SR) for muscle contraction have not been identified. In the present study, wild-type STIM1 and two STIM1 mutants (the Triple mutant, missing Ca^{2+}-sensing residues, and E136X, missing the C-terminus) were over-expressed in mouse primary skeletal myotubes. The wild-type STIM1 increased SOCE, while neither mutant had an effect on SOCE. Interestingly, the development of puncta by endogenous STIM1 and Orai1 was detected without any stimulus during the differentiation of myoblasts to myotubes, and increased puncta formation was observed in the triple mutant as well as the wild-type STIM1, suggesting that, in skeletal muscle, the formation of puncta is part of the differentiation process and not the necessary and sufficient condition for SOCE. On the other hand, the Triple mutant, but not E136X, decreased the Ca^{2+} release from the SR in response to KCl in a dominant-negative manner without affecting the SR Ca^{2+} amount or resting Ca^{2+} level. STIM1 was co-immunoprecipitated with the dihydropyridine receptor (DHRP). These results suggest that STIM1 could negatively regulate the Ca^{2+} release from the SR, possibly via its C-terminal interaction with DHPR.

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Mitsugumin 53 Attenuates the Activity of Sarcoplasmic Reticulum Ca^{2+}-ATPase 1a (SERCA1a) in Skeletal Muscle

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Mitsugumin 53 (MG53) is a member of membrane repair system in skeletal muscle. However, role(s) of MG53 in unique functions of skeletal muscle has not been addressed although MG53 is expressed only in skeletal and cardiac muscle. In the present study, MG53-binding proteins were searched among proteins mediating skeletal muscle contraction and relaxation using the binding assays of various MG53 domains and quadrupole time-of-flight mass spectrometry. MG53 binds to sarcoplasmic reticulum Ca^{2+}-ATPase 1a (SERCA1a) via its tripartite motif (TRIM) and PRY domains. The binding was confirmed in rabbit skeletal muscle and mouse primary skeletal myotubes by co-immunoprecipitation and immunocytochemistry. MG53 knock-down in mouse primary skeletal myotubes increased Ca^{2+}-uptake through SERCA1a (more than 35%) at micromolar Ca^{2+} but not at nanomolar Ca^{2+}, suggesting that MG53 attenuates SERCA1a activity possibly during skeletal muscle contraction or relaxation but not during the resting state of skeletal muscle. In-silico studies suggest that the binding of MG53 to SERCA1a is mediated by unique ways compared with bindings by other proteins containing TRIM or PRY domains.

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The Molecular Interactions of Heart Lim Protein (HLP) with RyR2 and Caveolin-3 is Essential for Effective Ca^{2+}-Induced Ca^{2+} Release in the Heart

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Heart LIM protein (HLP), a member of LIM-only protein family, has been abundantly detected in cardiac tissues, but its biological functions in the heart remain elusive. In the present study, using bacterial two hybrid screening and subsequent protein-protein interaction assays, we found that HLP directly interacts with RyR2 and caveolin-3 in the heart. Confocal and electron microscopy revealed that the interactions between the proteins are predominantly restricted to the subsarcolemma region of cardiomyocytes. Furthermore, knockdown of HLP impaired Ca^{2+}-induced Ca^{2+} release without directly affecting SR Ca^{2+} load and RyR2 activity. Taken together, our findings demonstrate that the adaptor function of HLP in the cell surface caveolae region is essential for efficient excitation-contraction coupling in the heart. (Supported by ''GIST Systems Biology Infrastructure Establishment Grant (2012)'' and by ''KISTI-KREONET'').

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Calcium Transients in Muscle Fibers Expressing Voltage-Sensitive Phosphoinositide Phosphatases

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Phosphoinositides play a role in a variety of cellular signaling processes; PtdIns(4,5)P_2 regulates the function of several types of ion channels in the plasma membrane and is the source of important second messengers. We tested the possible role of changes in the PtdIns(4,5)P_2 level in E-C coupling and Ca^{2+} homeostasis in mouse muscle fibers under voltage-clamp conditions. Measurements of indo-1 Ca^{2+} transients in fibers injected with PtdIns(4,5)P_2 revealed no significant changes in the voltage-dependence and maximum value for peak Ca^{2+} release. We then studied the consequences of the activation of a voltage-sensitive PtdIns-phosphatase (VSP). Expression of either Ci-VSP or Dr-VSP was achieved by in vivo electroporation. Confocal images of N-terminally EGFP-tagged Dr-VSP revealed a double-banded pattern of expression consistent with the triadic region and membrane current measurements from a depolarized holding potential showed the presence of a charge movement component consistent with the voltage-sensitive domain active in the t-tubule membrane. Rhod-2 Ca^{2+} transients generated by single depolarizing pulses within the voltage-dependent range of activation of Ca^{2+} release appeared unaffected by the presence of either VSP. In order to strongly activate the VSPs, fibers were depolarized by 10 successive 200 ms-long pulses from −80 mV to +80 mV; Ca^{2+} transients were compared to the ones elicited by an analogous series of pulses to +10 mV: on average, in control and Ci-VSP-positive fibers, the value for peak Ca^{2+} transient in response to the 10^{th} pulse to +80 mV was 94 ± 7 % (n=9) and 72 ± 6 % (n=7) the value in response to the corresponding pulse to +10 mV, respectively. The significant depression in Ci-VSP expressing fibers may be indicative that depletion of t-tubule PtdIns(4,5)P_2 can affect voltage-activated Ca^{2+} release. The work was supported by AFM, CNRS and Université Claude Bernard.

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Return of Myoplasmic Calcium (Ca) to Resting Levels following Stimulation of Fast- and Slow-Twitch Mouse Muscle Fibers

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To study the processes underlying the relaxation and recovery of skeletal muscle, we have used the high affinity fluorescent Ca indicators fluo-3 and fluo-4 to measure Ca transients in fibers from mouse fast-twitch (EDL) and slow-twitch (soleus) muscles (16 °C). Single fibers on the surface of small bundles of fibers were injected with indicator, and fluorescence changes (ΔF) were recorded for up to 60 s following a single action potential (AP). The full-duration at half-maximum (FDHM) of ΔF and the early decay time constant from 50% peak ΔF were larger in slow-twitch than in fast-twitch fibers (~220 and ~320 ms, respectively, vs. ~50 and ~70 ms). These findings are consistent with the larger FDHM of the Ca transient in slow-twitch fibers (Baylor and Hollingworth, J. Physiol., 2003). Interestingly, on